

UNCLASSIFIED

AD NUMBER

ADB210264

NEW LIMITATION CHANGE

TO

Approved for public release, distribution
unlimited

FROM

Distribution authorized to U.S. Gov't.
agencies only; Proprietary Info.; 28 Feb
96. Other requests shall be referred to
Commander, Army Medical Research and
Materiel Command, Attn: MCMR-RMI-S, Fort
Detrick, Frederick, MD 21702-5012.

AUTHORITY

19990609 - Ms Phylis M. Rhinehart, DCS
Info Mgmt, MCMR-RMI-S [70-1y], Ft Detrick,
MD .

THIS PAGE IS UNCLASSIFIED

AD _____

GRANT NUMBER: DAMD17-93-J-3071

TITLE: Effects of Diets High in Palmitoleic Acid on Serum Lipid Levels and Metabolism

PRINCIPAL INVESTIGATOR: J. David Curb, M.D.

CONTRACTING ORGANIZATION: University of Hawaii at Manoa
Honolulu, HI 96817

REPORT DATE: March 1996

TYPE OF REPORT: Final

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

DISTRIBUTION STATEMENT: Distribution authorized to U.S. Government agencies only (proprietary information, 28 Feb 96). Other requests for this document shall be referred to Commander, U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RMI-S, Fort Detrick, Frederick, MD 21702-5012.

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

19960517 165

REPORT DOCUMENTATION PAGE

*Form Approved
OMB No. 0704-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)			2. REPORT DATE	3. REPORT TYPE AND DATES COVERED	
			March 1996	Final (30 Sep 93 - 31 Jan 96)	
4. TITLE AND SUBTITLE			5. FUNDING NUMBERS		
Effects of Diets High in Palmitoleic Acid on Serum Lipid Levels and Metabolism			DAMD17-93-J-3071		
6. AUTHOR(S)					
J. David Curb, M.D.					
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)			8. PERFORMING ORGANIZATION REPORT NUMBER		
University of Hawaii at Manoa Honolulu, HI 96817					
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSORING/MONITORING AGENCY REPORT NUMBER		
Commander U.S. Army Medical Research and Materiel Command, Fort Detrick, Frederick, MD 21702-5012					
11. SUPPLEMENTARY NOTES					
12a. DISTRIBUTION/AVAILABILITY STATEMENT			12b. DISTRIBUTION CODE		
Distribution authorized to U.S. Government agencies only (proprietary information, 28 Feb 96). Other requests for this document shall be referred to Commander, U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RMI-S, Fort Detrick, Frederick, MD 21702-5012.					
13. ABSTRACT (Maximum 200 words)					
Using a controlled, crossover design feeding study, each of 30 healthy individuals (15 males and 15 females) aged 18 to 53 years, were fed, in random order, a tree nut based mono-unsaturated fat diet (MONO) (37% Kcal fat); an American Heart Association Step 1 diet (AHA) (30% Kcal fat); and a high saturated fat "typical American" diet (AMERICAN) (37% Kcal fat). Mean and (standard deviation) for total Cholesterol at the end of the AMERICAN was 201.2 mg/dl (30.4). That for the AHA and the MONO diets was 193.1 (34.5) and 191.3 (32.6) mg/dl. HDL was 55.3 (7.6), 52.0 (8.2) and 52.8 (8.2). Triglycerides were 77.5 (32.7), 83.6 (32.6) and 70.4 (26.0). In all cases the values after AHA and MONO diets had similar positive effects on lipids when compared to an AMERICAN diet. In an absorption study involving 6 males, the dietary fats in a nut oil based diet, as reflected by the rise in serum triglycerides, were more available than those using ground or whole nuts. None of the diets had a glycemic effect. These results suggest that high monounsaturated fat diets which incorporate nuts could be part of a healthy energy diet for combat troops.					
14. SUBJECT TERMS			15. NUMBER OF PAGES		
Cholesterol, Diet, Fats, Risk, Monounsaturated			37		
16. PRICE CODE					
17. SECURITY CLASSIFICATION OF REPORT		18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT	
Unclassified		Unclassified	Unclassified	Limited	

GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to *stay within the lines* to meet *optical scanning requirements*.

Block 1. Agency Use Only (Leave blank).

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

Block 3. Type of Report and Dates Covered.

State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C - Contract	PR - Project
G - Grant	TA - Task
PE - Program Element	WU - Work Unit Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. (If known)

Block 11. Supplementary Notes. Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement.

Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

DOD - See DoDD 5230.24, "Distribution Statements on Technical Documents."

DOE - See authorities.

NASA - See Handbook NHB 2200.2.

NTIS - Leave blank.

Block 12b. Distribution Code.

DOD - Leave blank.

DOE - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.

NASA - Leave blank.

NTIS - Leave blank.

Block 13. Abstract. Include a brief (*Maximum 200 words*) factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subjects in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code (*NTIS only*).

Blocks 17. - 19. Security Classifications. Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

FWL Where copyrighted material is quoted, permission has been obtained to use such material.

Govt Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

FWL Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

NA In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

NA For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

NA In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

NA In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

NA In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories. . . .

PI - Signature

Date

2/28/96

TABLE OF CONTENTS

<i>Introduction</i>	1
<i>Body</i>	3
<i>Conclusions</i>	13
<i>References</i>	14
<i>Appendix</i>	18
<i>Bibliography of all Publications and Meeting Abstracts</i>	32
<i>List of Personnel Receiving Pay From Grant</i>	33

INTRODUCTION

Diet has been shown to have significant effects on plasma lipids, lipoproteins, and coronary heart disease¹. Keys was among the first to report that populations with higher levels of fat in their diets had higher levels of coronary heart disease (CHD)². He and others later found that dietary saturated fatty acids are a major determinant of serum lipid levels and CHD risk³⁻⁴.

Traditionally, the apparently healthy inhabitants of regions like Greece and southern Italy have consumed relatively high fat diets containing substantial amounts of olive oil, a substance with high levels of the monounsaturated fatty acid, oleic acid. These "Mediterranean diets" frequently contain more calories from fat than the typical American diet but are higher in monounsaturated fats. Less than 10% of calories in such diets comes from saturated fats⁵.

Nuts also contain considerable amounts of monounsaturated fat and have also long been part of the traditional diet in many Mediterranean countries. Recent studies have shown potential beneficial effects of tree nuts in the diet⁶⁻⁸. The macadamia nut, a tree nut which originated in Australia, has become a primary export crop from Hawaii in recent years. The macadamia nut is approximately 75% fat by weight with 88% of its energy from fat. Monounsaturated fatty acids are the predominant fat. Oleic acid (18:1) is the predominant monounsaturate but a significant portion is palmitoleic acid (16:1) (Nutritional information from Genesis software, version 4.2, esha Research, Portland, Or.). Due to the high fat content of the macadamia nut, it has popularly been thought to be bad for health.

Since some nuts, including the macadamia nut, could be substituted for high saturated fat food items and therefore be a potential element in a healthy diet, it was felt to be valuable to investigate the effects of consuming a diet with a large percent of calories derived from macadamia nuts. We report here the results of carefully controlled crossover design feeding study comparing a 37% fat diet rich in macadamia nuts to a 37% fat "typical American" diet and a 30% Fat Step 1 diet conforming to the recommendations of the American Heart Association⁹.

Three types of studies are used to evaluate absorption and assimilation of dietary fats: fecal studies, isotope studies, or blood sampling over time after a test meal. Isotope studies are the most invasive and require special facilities, permits, personnel required, and are more expensive. Two types of test meals reported in studies of these factors: 1) primarily single food meals containing a high dose of fat (≥ 1 gram fat/kg body weight or 2) fats fed in a more usual combination with protein and carbohydrate (≤ 0.75 grams fat/kg body weight). The physical form of other nuts, such as the peanut, has been shown to alter the amount of fat absorbed¹⁰. Thus, a preliminary study to evaluate the lipemia postprandial changes as affected by a single meal containing different forms of macadamia nuts (whole, ground, or macadamia nut oil) in healthy human male subjects was carried out. Factors considered in the design of the study, including those factors which influence postprandial lipemia, are those reported to affect postprandial lipemia: total fat, type of dietary fatty acids, protein, carbohydrates, sucrose, total fiber and

types of fiber, mixed food meals or single food, gender, vigorous activity, prior diet, percentage of calories from fat, protein, or carbohydrate¹¹⁻³⁷.

BODY

METHODS

Feeding Study

Due to the scheduling of the kitchen for other purposes following the academic year and the fact that KCC students and staff would form part of the participant group, it was determined that the feeding study (Phase III) of the study would have to be accomplished during the academic semester, January - May 1994. Thus the more detailed absorption study (Phase I) was delayed in order to immediately focus on implementing Phase III. The meal testing phase (Phase II) was compressed somewhat and the participants recruited for Phase III were utilized to test meals in a buffet format and a slightly extended (6 day) run-in period.

Study Population

Through flyers, bulletins and newspaper advertisement, healthy, fit men and women of varied ethnicity and a broad age range were recruited. Eligibility criteria for participation in the study included 1) willingness to participate in a 3 month intensive dietary intervention; 2) a fasting cholesterol above 150 mg/dl and a triglyceride below 400 mg/dl and on no current pharmacologic treatment for hyperlipidemia; 3) weight between 80 to 130 percent of ideal weight; 4) no history of diabetes mellitus or pancreatic insufficiency, or an unstable medical condition of any kind; 5) no history of food allergies, especially to tree grown nuts; 6) not pregnant, breast feeding or taking certain birth control pills. To conform to an age range similar to that seen in the military, only individuals age between 18 and 55 years were eligible. In addition, in order to better approximate a military population, unlike many other feeding studies, efforts were made to include individuals with high levels of activity and thus high caloric intake.

Approximately 450 individuals were screened by telephone or in person. Of this number, twenty-five (25) men and twenty-eight (28) women took the initial blood test to determine if they met the cholesterol level requirements for the study. The forty-two remaining (23 men and 19 women) agreed to participate in the study. Three (3) individuals dropped out for other reasons. The 23 men and 19 women were enrolled in a six day run-in period to test their ability to stick to the diet as well as to give the kitchen staff practice with the complex cooking and exacting weighing requirements for the diets. Several individuals were eliminated in this initial period due to laboratory abnormalities. These included two men with abnormal liver enzymes, one man with very high triglyceride values and two men whose average cholesterol was below the study limit. One man was eliminated due to his decision to begin a weight-training program during the time period of the study and another man was eliminated due to an extremely high, and unexplained caloric requirement. One woman was eliminated due to her average cholesterol being below the study limit.

Overall twelve (12) subjects were dropped or withdrew prior to or during the initial run-in period; (8) in the initial study dietary period and (4) due to irregularities in their initial blood

results. The remaining 30 subjects (15 male and 15 female) were healthy, with no metabolic or endocrine diseases and with the exception of two subjects taking approved birth control pills, no subjects were taking regular prescription drugs. Subjects were generally students, professionals or both. This study was approved by the University of Hawaii institutional review board. All subjects gave written informed consent after thorough explanation of the study.

Experimental Design

The study was a controlled, crossover design feeding study of three 30-day dietary options. To minimize study group imbalance due to dropouts or exclusions during the run-in or early in the first dietary period, dietary assignments were randomly made in two phases. Both randomizations were stratified by gender. For the first dietary period, subjects were randomized to one of the three study diets (A, B, or C). Subsequently, during the later part of the first diet period subjects were randomized to the remaining diets they would follow during the next two periods. For example those who started with diet A were randomized to one of two possible sequences which would allow them to participate in all three diets ("B - C" or "C - B"). Similarly, subjects who started with diet B were randomized either to sequence "A - C" or "C - A". Subjects who started with diet C were randomized either to sequence "A - B" or "B - A". Thus randomization remained balanced throughout the study and all subjects had equal probability of being in each 3 diet sequence, despite several early dropouts. Study personnel involved in performing measurements and analyses were blinded to the diet sequences.

To avoid drop-outs the study design was carefully explained to all subjects by telephone as well as in individual and group meetings. Subsequently during a six-day run-in period to screen participants for compliance and willingness to accept the restrictions imposed by the dietary regimens, a 37% fat reference diet was served. This phase was followed by the three 30-day experimental periods.

The site of all meal preparation and consumption for the study was the 'Ohelo Food Services Instructional Building at Kapiolani Community College (KCC) under the auspices of the Food Service and Hospitality Education department. A new kitchen built for a master chef education program is being utilized for food preparation for the study. This kitchen is equipped with ovens, range tops, walk-in freezer and refrigerator. Small kitchen equipment and supplies were purchased by the study.

The subjects ate breakfast and dinner at the study site and were given a "bag" lunch each day prepared by study personnel. Breakfast was served to the study participants between 6:00 a.m. and 8:00 a.m., Monday through Friday. Lunches were picked up at breakfast, to be consumed at their discretion throughout the day. Dinner was served between 4:00 p.m. and 7:00 p.m., Monday through Friday. On Saturday, breakfast was served between 6:00 a.m. and 9:00 a.m. On Saturday, dinner was either given as a carry-out or the participants could choose to eat a dinner of their own selection, within guidelines established by the Nutrition Consultant on the study on the amount of fat consumed. On Sunday, brunch was served between 9:30 a.m. and 1:00 p.m. Dinner on Sunday was a carry-out meal.

Additional calories were made available in the form of "Unit" foods. These were in the form of 0.42 MJ (100 kcal) muffins or 0.42 MJ and 0.84 MJ (100 and 200 kcal) packages of chili. Unit foods were developed to match the nutrient profile for each diet. Participants were allowed to eat these "unit" foods ad libitum in addition to their diet regimen as long as they maintained their weight.

Daily energy intake needed to maintain weight was estimated for each subject according to Harris-Benedict equations by an activity factor¹³⁸ and compared to caloric intake on four-day food records completed immediately prior to the run-in period. These calculations were compared with body weight measurements and caloric levels were altered when necessary to maintain each subject's weight.

Subjects were requested to maintain their physical activity and other lifestyle habits constant. These factors were monitored through a daily diary provided by the study to record illness, medication use, and deviations from usual physical activity patterns or the study diet. To promote compliance, members of the study staff were available by pager 24 hours daily and deviations from the diet noted on food records were discussed with each participant in a non-confrontational manner.

The participants were each presented with a gift pack of donated items at the conclusion of the study. They also received a stipend of \$500.00. In addition, they were each given copies of the blood test results from the first blood draws during the study at the conclusion of the feeding study. If requested, copies of those blood test results were sent to their personal physicians.

Diets

Factors Affecting Diet Formulation

The following factors affected the formulation of the diets:

1. Nutrient information was required for any food and food product to be used in the study. Information for most raw food ingredients was obtained from ESHA's "GENESIS" ingredient database. All other nutrient information was obtained from the food manufacturer. Because the Nutritional Labeling Education Act would not be in place until May 8, 1994, many desirable products could not be used in the menu. Lack of adequate nutritional information required that essentially all foods be made from scratch including breads, muffins, and cookies.
2. Diets were initially designed using low-fat foods, food products, and recipes. In this way the appropriate fats could be added back to produce the correct fatty acid profile for each of the dietary regimes and kcal level.

3. Animal products had to be limited to prevent excessive cholesterol in the higher kcal diets.
4. To standardize the composition of all diets, each ingredient was weighed to 0.1 gram. This time-consuming procedure also eliminated certain recipes from the menu.
5. Foods offered had to be acceptable to the local palate of the subjects, if they would be expected to comply throughout the whole study.
6. Once a week on Saturday nights, subjects had the choice to take foods prepared by the study personnel or eat out with restrictions limiting the amount of fat consumed. Therefore all nuts were incorporated into their breakfast in the form of a fruit "smoothie" (berries, milk, and nuts/oils). Approximately half of the subjects took the Saturday evening meal.
7. A limited amount of non-caloric caffeinated beverages were allowed (up to five (5) cups of coffee/day); all other drinks were required to be non-caloric decaffeinated. Consumption of any beverage is listed on the daily diary.
8. Up to five (5) alcoholic drinks (wine, beer, or whiskey) were allowed per week; no more than two (2) drinks on any one (1) night. No alcohol was allowed within five (5) days of blood drawing. Consumption of alcoholic beverages was recorded on the daily diary.

The diets utilized were a "typical American" diet, a macadamia nut high monounsaturated fat diet and an American Heart Association Step 1 "Prudent" diet (Table 1). A 10 day cycle menu was designed with Genesis Ingredient database (ESHA Research) using whole foods to match the nutrient profile shown in Table 1. All three diets were designed to contain 17% of total energy from protein with the percent of energy from carbohydrate and fat depending on the diet. Polyunsaturated fatty acids and cholesterol content of all diets were kept constant. Intake of salt and pepper was not restricted. The foods were prepared using recipes and methods similar to those commonly used (except for weighing ingredients to 0.1gm) but in which the experimental diets were adapted to contain modified amounts of the appropriate foods and nutrients. The foods in the high monounsaturated fatty acid diet were similar to those in the "typical American" diet with reduced portions of fatty foods and visible fats, some or all of which were replaced by finely ground macadamia nuts in various recipes designed to obscure the identity of these ingredients. This was not uniformly successful due to a slight grit mouth feel and relatively high volume of the ground nuts. However, to avoid inter-individual variability due to differences in mastication or chewing, the uniform fine grind of the nuts was felt to be important because digestibility and absorption of peanuts has been shown to be affected by the grind of the nut¹⁰.

Measurements

Before breakfast and after a 12 to 14 hour fast, each subject had blood drawn on three days at the end of the run-in period and three days at the end of each dietary period. No alcoholic beverages were allowed in the five days prior to a blood draw. Analyses for total cholesterol, HDL cholesterol, and triglycerides were carried out at the Medlantic Laboratory in one batch after the end of study utilizing serum specimens frozen at -70 degrees centigrade for 3 to 6 months³⁹. Cholesterol was determined by the Able-Kendell method and HDL was determined directly after MnCl-heparin precipitation. The laboratory was standardized according to the Lipid Standardization Program of the Centers for Disease Control and Prevention and the National Heart Lung and Blood Institute. Values for LDL cholesterol were calculated by subtraction utilizing the Friedewald algorithm. In order to reduce the impact of interindividual variability, the average of the three daily values at each time period was used for statistical calculations. For each of the three dietary treatments chemical analyses were conducted on homogenized samples of four complete days of the 10-day cycle menu (n=12). Chemical analyses were conducted by Food Products Laboratory, Portland, Oregon.

Body weight measurements were taken to the nearest tenth of a pound on a UC 300 digital Health Scale at the feeding site prior to breakfast in street clothes, without shoes or heavy clothing. Weight was measured daily during the run-in period and the first two weeks of the intervention. Subsequently it was measured two times per week. Skinfold measurements were made at the beginning and end of each diet period with Lange skinfold calipers. Using chest, abdomen and thigh skinfolds in men and triceps, suprailiac and thigh skinfolds in women, percent body fat was calculated⁴⁰⁻⁴¹.

Statistical Analyses

Statistical methods included linear models for the analysis of a three period cross-over design⁴². Such models included parameters for assessing the influence of diet, period and carry-over effects into subsequent dietary periods. Transforming the data using log transformations had no affect on the findings reported here. All reported p-values were based on two-sided tests of significance.

Absorbtion Study

Subject Recruitment

Six (6) healthy adult male volunteers were recruited from prior participants of the Diamond Head Nutrition Research 3-month feeding study (See Subject Table). Informed consent was acquired from all participants and a Dietary History Data form (24-hour diet record) was given to each subject one week prior to study. This diet record was collected prior to the first test day or at the latest on the day of the first blood drawing.

Subjects taking medications which might interfere with lipid metabolism or with plasma cholesterol or triglyceride (TG) concentrations were excluded, as well as diabetics-juvenile or adult onset. All subjects were within normal Body Mass Index ($BMI \leq 27.8$).

Treatment

Each subject served as his own control. Test meals and subsequent blood tests were administered 7 days apart. Subjects were instructed not to deviate from regular habits and to avoid alcohol consumption for three (3) days prior the test meal as well as to limit strenuous exercise the day prior to each test. Participants were instructed to refrain from food and liquid for 12-14 hours immediately before the morning of the test meal. Subjects were allowed to read, study, watch videos, or use a computer during the experiment, but were not allowed to leave clinic. Movement during test days was kept to a minimum until the last blood draw.

Test Meals

Tests meals were similar (containing 45 grams of macadamia nuts) with the exception of the macadamia nut form (whole, finely crushed, or oil). In the test meal using macadamia nut oil (33.3 g), egg white and Kellogg's all bran fiber was used to make up the 11.7g difference. Test meals consisted of commercially available foods: hot oatmeal (Quaker Oats. Barrington, IL) with skim milk, fruit, Saffola stick margarine and sugar on top, one extra large egg, salt, pepper, french bread, and skim milk. The bread was used to clean the bowl of all food.

Using ESHA's Genesis database, each meal was calculated to provide 46% energy as carbohydrate, 17% as protein, and 37% as fat, with approximately 225 mg cholesterol and a ratio of polyunsaturated to saturated fatty acids (P:S ratio) of 0.35. Total dietary fiber was 13.1 g. Each test meal provided approximately 1010-1030 kcal, one-third the estimated daily energy requirement for these male subjects (3,100 to 3,500 kcal as determined by a 3-month feeding trial).

On any test day, 2 subjects were served the whole nut test meal, 2- the ground nut test meal and 2- the nut oil test meal. Throughout the three (3) test days, subjects only consumed each of the test meals once.

Meal Preparation

All foods and meals were purchased and prepared by an individual trained in food handling and food preparation for nutritional studies. Foods were weighed to 0.1 gram and assembled on the morning of the study. An additional test meal (containing the whole, ground, and oil form) was homogenized and sent for chemical analyses (proximate, cholesterol and fatty acid profile saturated, polyunsaturated and monounsaturated as well as individual fatty acids), and dietary fiber (soluble and insoluble)).

Blood Sampling

Prior to the test meal days, an initial screening CBC and Chem profile 20 was completed to assure that each participant still met the study criteria. Blood analyses during the study were total triglycerides (TG), total cholesterol, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) by calculation, and glucose.

A wrist or hand vein was catheterized with an infusion set and a fasting, baseline blood sample was obtained. Blood was drawn at -5 minutes, 1, 2, 3, 4, 5, 6, 7, and 8 hrs after the meal. After cannulation and each blood draw, 1 cc of saline was used to flush the cannula. Prior to each blood sample taken, a 2 cc blood sample was discarded to avoid hemodilution with sterile normal saline present at the site of sampling. Participants were restricted from food intake throughout the test day. However, free access to water during the test day was allowed, but always measured and given by coordinator.

RESULTS

Feeding Study

Fifty-three percent of the participants who completed the feeding study were Caucasian, thirty-seven percent were Asian-Pacific Islanders and ten percent were black. Ages ranged between 19 and 54 years with a mean age of 36.7 years men and 33.8 years for women (Table 2). The mean body mass index of the males was 24 ± 2.4 (range 19.5-27.9). The mean body mass index for the females was 22 ± 2.6 (range 19.1-28.3). Only one subject had over a 3 pound weight gain or loss during the study period (3.4 lbs). No differences in skinfold thickness were seen throughout the study. Only one of the participants smoked. The mean baseline cholesterol ranged from normal to high. However, the majority of participants did not meet clinical criteria for hyper-cholesterolemia. In males the mean baseline cholesterol was 5.26 mmol/L (203.5 mg/dl) and ranged from 3.9 to 6.9 mmol/L (158 to 267 mg/dl). In females the mean was 5.3 mmol/L (206 mg/dl) and ranged from 4.1 to 7.0 mmol/L (157 to 272 mg/dl). Triglycerides tended to be relatively low and HDL's relatively high compared to average American values. Kilocalorie requirements for subjects ranged from 6.3 MJ to 14.7 MJ (1500 kcals to 3500 kcals) for females and 10.5 to 16.7 MJ (2500 to 4000 kcals) for males, somewhat higher than the range reported in many studies. A comparison between the actual macronutrient profiles expressed as percent of calories consumed and that formulated using Genesis software is shown in Table 3. The differences between the values were minimal.

Shown in Table 4 are the mean and standard deviation for lipid and lipoprotein factors at the end of each dietary period for the overall group and for the two sexes. For both sexes combined, mean total cholesterol at the end of completion of the diet treatments was significantly higher ($p < 0.01$) for the typical American diet than for the other two diet treatments. The mean LDL cholesterol was also higher for the typical American diet ($p < 0.5$). Mean

triglyceride values were significantly different from the typical American diet ($p < 0.05$) for both of the other diets. The mean HDL was lower after the Step 1 ($p < 0.001$) and the Monounsaturated fat diet ($p < 0.01$). The patterns were similar in both sexes although the differences were not consistently significant. The smaller sample sizes and potentially greater variability contributed to these findings.

Figure 1 shows mean cholesterol trends for each of the six randomization sequences. There was a tendency for total cholesterol to drop through the study period, in all randomization groupings, a phenomenon which is common in individuals who become involved in studies in which their diets are controlled to greater extent than in a free living environment. These trends did not exceed expectations and there were no significant carry-over effects between dietary periods.

Shown in Table 5 are the results of the additional analyses of lipoproteins and glucose, insulin and c-peptide. As can be seen, for the apolipoproteins A-I and A-II as well as for LDL particle size, the results were similar to those for cholesterol with lower values after the AHA and high monounsaturated fatty acid diets. For lipoprotein a [Lp(a)], the high fat diet had the lowest values as might be expected. Fasting glucose levels were lowest after the AHA diet and fasting insulin levels were highest after the monounsaturated fat diet but the differences were small and probably not clinically significant. C-peptide values were not different between diets.

Absorption Study

The mean of the three baseline values for fasting glucose for the men in the absorption study varied between 75.7 and 99.0 (Table 6). The mean for cholesterol varied between 176.3 and 272.3. For triglyceride the values ranged from 54.7 to 165.0. The serum mean serum glucose for all men combined at each time point at which blood was drawn is shown in Table 7. For ground nuts, whole nuts and the oil there was a decrease in serum glucose until about 3 hours after the meal but the baseline levels had still not been reached after 8 hours. The patterns over time were significant ($p=0.0018$). For serum cholesterol the patterns over time were not marked and were not significant (Table 8). Serum triglyceride rose significantly for all diets, peaking at the third or fourth hour. The rise was greatest for the oil diet and least for the whole nut diet, while it was intermediate for the ground nut diet. The mean triglyceride fell after reaching its peak and this fall was most marked in the whole nut diet.

DISCUSSION

The three diets utilized in the feeding study were designed to evaluate both substitution of monounsaturated fat (primarily derived from macadamia nuts) or saturated fat, as well as to compare the high monounsaturated fat diet to the lower fat American Heart Association Step 1 diet. The findings presented here indicate that the monounsaturated fat nut based high (37%) fat diet and the moderately low (30%) fat American Heart Association Step 1 diet had similar effects on lipid profiles. The results suggest that the monounsaturated fats present in macadamia

nuts have a favorable effect on serum cholesterol levels of healthy adults when eaten in place of saturated fats. It should be noted that this effect was seen despite the fact that the study included a wide range of ethnic groups, had a broad age range, had a relatively short 30 day period on each diet, and included only relatively lean, healthy individuals of both sexes, many of whom had relatively low cholesterol levels. It is of interest that the results of the study are similar in men and women. Whether these findings will be applicable to older individuals, those with hypercholesterolemia or obese individuals can not be determined from the data available. It is possible that the differences seen here would be increased with a longer period for each diet.

Making direct comparisons between studies is difficult, due to widely variable differences in the length of studies, the characteristics of the participants, as well as the range of fat, cholesterol and fiber contents of diets used in the various studies. However, the results of this study are generally consistent with those seen in other studies contrasting high monounsaturated diets to high saturated fat and low fat diets⁴³⁻⁵⁰. The magnitude of the decrease of serum cholesterol by both the moderately low fat and the high monounsaturated fat diets in this study is lower than in some studies. On the other hand, the decrease in triglycerides with the monounsaturated fat diet is larger than reported in most other similar studies. The 4.5% lower HDL with the monounsaturated fat diet compared to the high fat diet is consistent with the changes seen in other published studies which report anywhere from a 2% increase to a 6% drop. Hegsted has pointed out that the effects of diet on HDL are complex and may not be subject to meaningful interpretation⁴. Thus interpretation of the HDL results of the present study may also be difficult. In general the changes in HDL induced by diet are relatively small, the day to day variation is large and the clinical significance of such changes is not known⁵¹. Further investigation of HDL effects in a longer term study is warranted. Given the data available on the effects of monounsaturated fats in the diet and the nutrient composition of the macadamia nut, a beneficial effect could be hypothesized. However, nuts are complex foods which contain many nutrients and macadamia nuts have a fatty acid profile which differs somewhat from most common sources of monounsaturated fatty acids in the diet. One of these monounsaturated fatty acids, palmitoleic acid, was reported to raise cholesterol in one study⁵². The results of the current study give no support to such an effect, since, as in other studies the magnitude of the effect of a diet high in monounsaturated fatty acids is similar to that of a low fat diet.

An epidemiologic study of California Adventists was one of the first to suggest the potential health benefits of nuts⁵⁴. That study suggested that regular consumption of nuts had a protective effect against coronary heart disease in that population. Dietary studies of the walnut and the almond have provided more specific evidence of the potential cholesterol lowering properties of this group of foods⁶⁻⁸. As with all high fat foods, nuts may be a significant source of calories, and if not substituted for other fatty foods they could result in weight gain. However, in a pilot study for the present study in which 70 healthy free living¹²

¹²unpublished data

subjects were randomized to groups given supplements of 3.2 ounces (600 calories) or 1.6 ounces (300 calories) of macadamia nuts as a supplement, or to a regular diet group, there was no significant change in the mean weight of any of the groups after one month⁵⁴. All groups received only a single 15 minute dietary counseling session on food substitutions to avoid weight gain and eat a healthy diet. Some participants in that study reported a suppression of appetite after eating their nuts each day. Eating nuts may be associated with increased satiety but little data is available. No important side effects to consistent ingestion of large amounts of macadamia nuts were noted in either the pilot study or the feeding study. In the pilot study gastrointestinal discomfort consistent with those experienced with radical shifts in dietary fat content were not uncommon but usually temporary. There was no difference in serum cholesterol between the groups in the pilot study although the high dose macadamia group ate 50 percent of their calories as fat.

In the absorption study, as expected, the dietary fats in the oil diet, as reflected by the rise in serum triglycerides, were most available in the oil diet. This is consistent with absorption studies using peanut products¹⁰. A slower decline in triglycerides might have been interpreted after the oil diet due to the lower fiber content³⁶. The fiber content does not, however explain the slower fall after the ground nut diet. The study demonstrated a marked effect on serum glucose.

As shown in other studies none of the diets had a short term effect on serum cholesterol⁵⁵. None of the diets demonstrated a glycemic effect and in fact in all three the glucose fell significantly.¹³

¹³unpublished data

CONCLUSIONS

In conclusion, the results of this study indicate that the consumption of significant amounts of macadamia nuts in place of other dietary saturated fats appears to lower serum cholesterol when total fat intake and caloric intake are maintained at stable levels. In addition the effect of the monounsaturated fat diet on cholesterol and other lipids was as favorable as that seen with a lower fat AHA Step 1 diet. These results coupled with the palatability of macadamia nuts suggest that their consumption could be part of a healthy diet.

Further investigation of the use of energy dense dietary products prepared from natural products available in the United States and high in the monounsaturated fatty acid for use by the men and women of our armed forces in combat situations is warranted. The development of acceptable energy dense dietary products for use in field and combat situations would simplify logistics while providing greater flexibility. Monounsaturated fatty acids have been demonstrated to have the potential of providing high energy density without the consequences of atherosclerosis and increased cancer risk associated with other high fat diets. Palmitoleic acid appears to be especially promising in this regard, and palatable food products high in this fatty acid are produced in the United States. Longer term investigations which test diets which better approximate the typical garrison diet and would be adaptable for inclusion in Meals Ready to Eat (MRE) based diets should be carried out. Continued exploration of diets consumed in a free living environment, with increased emphasis on meals consumed away from the study site and over longer periods are needed.

With the experience of this study, the investigators would now recommend that similar future studies have at least a two week run-in period. At this point a less complex study with two diets, a high saturated fat diet similar in fat content to the average MRE and garrison diet (37% fat) and a diet of similar fat content (37%) but with emphasis on monounsaturated fats (21%) over at least a 10 week period each would be desirable.

The development of healthier, yet acceptable energy dense dietary products for use in field and combat situations as Meals Ready to Eat (MRE) would contribute to simplified logistics while providing greater potential long term fitness. Such products might be especially applicable for Rations Lightweight (RLW) and for restricted Rations MRE's.

REFERENCES

1. Kris-Etherton PM, Krummel D, Dreon D, Makey S, Wood PD. The effect of diet on plasma lipids, lipoproteins, and coronary heart disease. *J Am Diet Assoc*, 1988;88:1373-1400.
2. Keys A, Atherosclerosis: a problem in newer public health. *J Mt Sinai Hosp* 1953;20:118-139.
3. Keys A, Ed. 1970. Coronary heart disease in seven countries. *Circ* 41 (Sup 1) 759.
4. Hegsted DM, Ausman LM, Johnson JA, Dallal GF. Dietary fat and serum lipids: an evaluation of experimental data. *Am J Clin Nutr* 1993;57:875-883.
5. Willett WC, Sacks F, Trichopoulou A, Drescher G, et al. Mediterranean diet pyramid: a cultural model for healthy eating. *Am J Clin Nutr* 1995;61:1402s-1406s.
6. Spiller GA, Jenkins DJA, Cragen LN, Gates JE, Bosello O, Berra K, Rudd C, Stevenson M, Superko R: Effects of a diet high in monounsaturated fat from almonds on plasma cholesterol and lipoproteins. *J Am Coll Nutr* 1992, 11;126-130.
7. Sabate J, Fraser GE, Burke K, Knutson SF, Bennett H, Lindsted KD. Effects of Walnuts on Serum Lipid Levels and Blood Pressure in Normal Men. 1993, *NEJM* 328:603-607.
8. Abbey M, Noakes M, Belling GB, Nestel PJ. Partial replacement of saturated fatty acids with almonds or walnuts lowers total plasma cholesterol and low-density-lipoprotein cholesterol. 1994, *Am J Clin Nutr* 59:995-999.
9. Nutrition Committee, American Heart Association: Dietary guidelines for healthy American Adults. *Circulation* 77:721A,1988.
10. Levine-AS; Silvis-SE. Absorption of whole peanuts, peanut oil and peanut butter. *New England J Med.* 1980 Oct 16; 303:917-918.
11. Brown-AJ; Roberts-DC. Moderate fish oil intake improves lipemic response to a standard fat meal. A study in 25 healthy men. *Arterioscler-Thromb.* 1991 May-Jun; 11(3): 457-66.
12. Caputo-FA; Mattes-RD. Human dietary responses to perceived manipulation of fat content in a midday meal. *Int-J-Obes-Relat-Metab-Disord.* 1993 Apr; 17(4): 237-40.
13. Cara-L; Dubois-C; Borel-P; Armand-M; Senft-M; Portugal-H; Pauli-AM; Bernard-PM; Lairon-D . Effects of oat bran, rice bran, wheat fiber, and wheat germ on postprandial lipemia in healthy adults. *Am-J-Clin-Nutr.* 1992 Jan; 55(1): 81-8.
14. Cohn-JS; McNamara-JR; Schaefer-EJ. Lipoprotein cholesterol concentrations in the plasma of human subjects as measured in the fed and fasted states. *Clin-Chem.* 1988 Dec; 34(12): 2456-9.
15. Cohn-JS; Johnson-EJ; Millar-JS; Cohn-SD; Milne-RW; Marcel-YL; Russell-RM; Schaefer-EJ. Contribution of apoB-48 and apoB-100 triglyceride-rich lipoproteins (TRL) to postprandial increases in the plasma concentration of TRL triglycerides and retinyl esters. *J-Lipid-Res.* 1993 Dec; 34(12): 2033-40.
16. Crisman Mitchell-D, McMahon-KE, Shively-CA, Apgar-JL, and Kris-Etherton-PM. Digestibility of cocoa butter and corn oil in human subjects: a preliminary steady. *Am J Clin Nutr* 1989;50:983-6.
17. Cunningham-KM and Read-NW. The effect of incorporating fat into different components of a meal on gastric emptying and postprandial blood glucose and insulin responses. *Brit-J-Nutr.* 1989. 61:285-290.
18. DeLany-JP; Vivian-VM; Snook-JT; Anderson-PA. Effects of fish oil on serum lipids in men during a controlled feeding trial. *Am-J-Clin-Nutr.* 1990 Sep; 52(3): 477-85.
19. Dubois-C; Cara-L; Armand-M; Borel-P; Senft-M; Portugal-H; Pauli-AM; Bernard-PM; Lafont-H; Lairon-D. Effects of pea and soybean fibre on postprandial lipaemia and

- lipoproteins in healthy adults. *Eur-J-Clin-Nutr.* 1993 Jul; 47(7): 508-20.
20. Edelstein-C; Frederich-C; Schuelke-JC; Jensen-WE; Sitrin-M; Iverius-PH; Scanu-AM. Hypoalphalipoproteinemia: postprandial response of subjects with preprandial normotriglyceridemia and hypertriglyceridemia to various diets. *Metabolism.* 1993 Feb; 42(2): 247-57.
21. Grant-KI; Marais-MP; Dhansay-MA. Sucrose in a lipid-rich meal amplifies the postprandial excursion of serum and lipoprotein triglyceride and cholesterol concentrations by decreasing triglyceride clearance. *Am-J-Clin-Nutr.* 1994 Apr; 59(4): 853-60.
22. Griffiths-AJ; Humphreys-SM; Clark-ML; Fielding-BA; Frayn-KN. Immediate metabolic availability of dietary fat in combination with carbohydrate. *Am-J-Clin-Nutr.* 1994 Jan; 59(1): 53-9.
23. Hartung-GH; Lawrence-SJ; Reeves-RS; Foreyt-JP. Effect of alcohol and exercise on postprandial lipemia and triglyceride clearance in men. *Atherosclerosis.* 1993 Apr; 100(1): 33-40.
24. Harris-WS; Connor-WE; Alam-N; Illingworth-DR. Reduction of postprandial triglyceridemia in humans by dietary n-3 fatty acids.. *J-Lipid-Res.* 1988 Nov; 29(11): 1451-60.
25. Jones-PJ; Leitch-CA; Pederson-RA. Meal-frequency effects on plasma hormone concentrations and cholesterol synthesis in humans. *Am-J-Clin-Nutr.* 1993 Jun; 57(6): 868-74.
26. Karpe-F; Tornvall-P; Olivecrona-T; Steiner-G; Carlson-LA; Hamsten-A. Composition of human low density lipoprotein: effects of postprandial triglyceride-rich lipoproteins, lipoprotein lipase, hepatic lipase and cholesteryl ester transfer protein. *Atherosclerosis.* 1993 Jan 4; 98(1): 33-49.
27. Miller-M; Kwiterovich-PO Jr; Bachorik-PS; Georgopoulos-A. decreased postprandial response to a fat meal in normotriglyceridemic men with hypoalphalipoproteinemia. *Arterioscler-Thromb.* 1993 Mar; 13(3): 385-92.
28. Redard-CL; Davis-PA; Schneeman-BO. Dietary fiber and gender: effect on postprandial lipemia. *Am-J-Clin-Nutr.* 1990 Nov; 52(5): 837-45.
29. Rifai-N; Merrill-JR; Holly-RG. Postprandial effect of a high fat meal on plasma lipid, lipoprotein cholesterol and apolipoprotein measurements. *Ann-Clin-Biochem.* 1990 Sep; 27 (Pt 5): 489-93.
30. Salomaa-V; Rasi-V; Pekkanen-J; Jauhainen-M; Vahtera-E; Pietinen-P; Korhonen-H; Kuulasmaa-K; Ehnholm-C. The effects of saturated fat and n-6 polyunsaturated fat on postprandial lipemia and hemostatic activity. *Atherosclerosis.* 1993 Oct; 103(1): 1-11.
31. Schneeman-BO; Kotite-L; Todd-KM; Havel-RJ. Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans. *Proc-Natl-Acad-Sci-U-S-A.* 1993 Mar 1; 90(5): 2069-73.
32. Surina-DM; Langhans-W; Pauli-R; Wenk-C SO . Meal composition affects postprandial fatty acid oxidation. *Am-J-Physiol.* 1993 Jun; 264(6 Pt 2): R1 065-70 .
33. Svaneborg-N; Moller-JM; Schmidt-EB; Varming-K; Lervang-HH; Dyerberg-J. The acute effects of a single very high dose of n-3 fatty acids on plasma lipids and lipoproteins in healthy subjects. *Lipids.* 1994 Feb; 29(2): 145-7.
34. Traianedes-K, Collier-GR, O'Dea-K. Ingestion of different types of fat in the evening meal does not affect metabolic responses to a standard breakfast. *Am J Clin Nutr* 1990;52:442-5.

35. Williams-CM; Moore-F; Morgan-L; Wright-J. Effects of n-3 fatty acids on postprandial triacylglycerol and hormone concentrations in normal subjects. *Br-J-Nutr.* 1992 Nov; 68(3): 655-66.
36. Van Amelsvoort-JMM, Van Stratum-P, Kraal-JH, Lussenburg-RN and Houtsmuller-UMT. Effects of varying the carbohydrate:fat ratio in a hot lunch on postprandial variables in male volunteers. *Brit J Nutr.* (1989), 61, 267-283.
37. Van-Amelsvoort-JM; Van-Stratum-P; Kraal-JF; Lussenburg-RN; Dubbelman-GP. Minor difference in postprandial responses of men between starch and sugar when replacing fat in a normal meal. *Br-J-Nutr.* 1990 Jan; 63(1): 37-51.
38. Harris JA, Benedict . A Biometric study of basal metabolism in man. Washington, D.C.; Carnegie Institute of Washington; 1919, publication 279.
39. Laboratory Methods and Quality Assurance in the Cardiovascular Health Study. *Clin Chem* 1995;41:264-270.
40. Jackson AS, Pollack ML. Generalized equations for predicting body density of men. *Brit J Nutr* 1978;40:497-504.
41. Jackson AS, Pollack ML. Generalized equations for predicting body density of women. *Med Sci Sports Exerc* 1980;12:175-182.
42. Winer BJ. Statistical Principals for Experimental Design. McGraw-Hill, New York, 1971.
43. Colquhoun DM; Moores D; Somerset SM; Humphries JA. Comparison of the effects on lipoproteins and apolipoproteins of a diet high in monounsaturated fatty acids, enriched with avocado, and a high-carbohydrate diet. *Am J Clin Nutr* 1992;56:671-677.
44. Berry EM; Eisenberg S; Friedlander Y; Harats D; Kaufmann NA; Norman Y; Stein Y. Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins-the Jerusalem Nutrition Study. II. Monounsaturated fatty acids vs carbohydrates. *Am J Clin Nutr* 1992;56:394-4035.
45. Grundy SM; Florentin L; Nix D; Whelan MF. Comparison of monounsaturated fatty acids and carbohydrates for reducing raised levels of plasma cholesterol in man. *Am J Clin Nutr* 1988;47:965-969.
46. Grundy SM. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. *N Engl J Med* 1986;314:745-748.
47. Mensink RP; Katan MB. Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. *Lancet* 1987;1:122-125.
48. Mensink RP, de Groat MJM, van de Broeke LT; Severijnen-Nobels AP; Demacker PNM, KatAN MB. Effects of monounsaturated fatty acids complex and carbohydrates on serum lipoproteins and apoproteins in healthy men and women. *Metabolism* 1989;38:172-178.
49. Baggio G; Pagnan A; Muraca M; Martini S; Opportuno A; Bananome A, Ambrosio GB, Ferrari S, Guarini P, Piccolo D, Manzato E, Corrocher R, Crepaldi G. Olive-oil-enriched diet: effect on serum lipoprotein levels and biliary cholesterol saturation. *Am J Clin Nutr* 1988 Jun;47(6):960-964.
50. Ginsberg HN; Barr SL; Gilbert A; Karmally W; Deckelbaum R, Kaplan K, Ramakrishnan R, Holleran S, Dell RB. Reduction of plasma cholesterol levels in normal on an American Heart Association Step 1 diet or a Step 1 diet with added monounsaturated fat. *N Engl J Med* 1990 Mar 1;322(9):574-579.
51. Hayes KC, Khosla P. Dietary fat thresholds and cholesterolemia. *FASEB J* 1992;6:2600-2607.

52. Nestel P, Clifton P, Noakes M. Effects of increasing palmitoleic acid compared with palmitic and oleic acids on plasma lipids of hypercholesterolemic men. *J Lipid Res* 1994;35:656-662.
53. Fraser GE, Sabate J, Beeson WL, Strahan M. A possible protective effect of nut consumption on risk of coronary heart disease. *Arch Intern Med* 1992;152:1416-1424.
54. Curb, J.D., Wergowske, G.L. Hankin, J. "The effect of dietary supplementation with macadamia nuts on serum lipid levels in humans." Proceedings of the First International Macadamia Research Conference, Kona, Hawaii, 1992.
55. Ng-TK. Blood cholesterol screening: influence of fasting state, biological variation and the single cholesterol assay on total cholesterol level. *Med-J-Malaysia*. 1993 Mar; 48(1): 12-6.

APPENDIX

Table 1. Nutrient composition profile of the three study diets

	Typical American	AHA Step 1	HIGH MONOUNSATURATED
	% of Kcals	% of Kcals	% of Kcals
Protein	17	17	17
Carbohydrate	46	53	46
Total Fat	37	30	37
Saturated Fat	16	9	9
Polyunsaturated Fat	7	7	7
Monounsaturated Fat	14	14	21

¹⁶¹⁶unpublished data

Table 2 - Mean and standard deviation of baseline characteristics of study subjects by sex

	Males (N=15)		Females (N=15)	
	Mean	SD	Mean	SD
Age (years)	36.7	9.1	33.8	10.6
Body Mass Index (BMI)	24.0	2.4	22.0	2.6
Cholesterol mmol/L (mg/dl)	5.29 (204.5)	0.88 (34.1)	5.32 (205.6)	0.87 (33.8)
LDL mmol/L (mg/dl)	3.50 (135.2)	0.73 (28.4)	3.43 (132.8)	0.83 (32.0)
HDL mmol/L (mg/dl)	1.39 (53.6)	0.18 (6.8)	1.46 (56.5)	0.18 (6.8)
Triglyceride mmol/L (mg/dl)	2.62 (78.3)	0.80 (30.8)	2.13 (82.2)	0.94 (36.3)

17

¹⁷unpublished data

Table 3 - Energy content and nutritional profile of macro-nutrients as planned and as observed from chemical analyses of 12 complete day samples

NUTRIENT	TYPICAL AMERICAN		AHA		HIGH MONOUNSATURATED	
	Planned	Observed	Planned	Observed	Planned	Observed
Protein	17	17	17	16	17	17
Carbohydrate	46	48	53	54	46	48
Total Fat	37	35	30	30	37	35
Saturated	16	14	9	8.6	9	8.8
Polyunsaturated	7	8.8	7	6.7	7	6.5
Monounsaturated	14	12	14	14.7	21	19.8
Energy MJ (kcal)	13.4 (3208)	13.8 (3285)	13.8 (3293)	14.3 (3423)	13.7 (3280)	14.3 (3415)
Cholesterol mg	300	305	300	297	300	300

Diets were formulated and nutrients calculated using Genesis Software Vers. 4.2 from ESHA Research.¹⁸

¹⁸unpublished data

Table 4. Mean serum levels of lipids and lipoproteins at the end of each dietary period for all subjects combined and by gender.

LIPID	TYPICAL AMERICAN		AHA		HIGH MONOUNSATURATED	
	MEAN	SD	MEAN	SD	MEAN	SD
Cholesterol mmol/L (mg/dl)	5.20 (201.2)	0.79 (30.4)	4.99** (193.1)	0.89 (4.5)	4.95** (191.3)	0.84 (32.6)
LDL mmol/L (mg/dl)	3.37 (130.4)	0.66 (25.7)	3.21* (124.3)	0.79 (30.4)	3.22* (124.4)	0.76 (29.5)
Triglyceride mmol/L (mg/dl)	2.00 (77.5)	0.85 (32.7)	2.16* (83.6)	0.84 (32.6)	1.82* (70.4)	0.67 (26.0)
HDL mmol/L (mg/dl)	1.43 (55.3)	0.20 (7.6)	1.34*** (52.0)	0.19 (7.2)	1.37** (52.8)	0.21 (8.2)
<u>MALES</u>						
LIPID	MEAN	SD	MEAN	SD	MEAN	SD
Cholesterol mmol/L (mg/dl)	5.26 (203.5)	0.71 (27.6)	5.01* (193.7)	0.86 (33.2)	5.04 (195.0)	0.73 (28.3)
LDL mmol/L (mg/dl)	3.47 (134.2)	0.58 (22.4)	3.28* (126.8)	0.71 (27.3)	3.40 (131.5)	0.64 (24.7)
Triglyceride mmol/L (mg/dl)	2.12 (82.0)	0.58 (36.3)	2.32 (89.8)	0.95 (36.8)	1.81* (70.0)	0.75 (28.9)
HDL mmol/L (mg/dl)	1.37 (52.8)	0.24 (9.1)	1.26*** (48.9)	0.21 (8.0)	1.28** (49.5)	0.23 (9.0)
<u>FEMALES</u>						
LIPID	MEAN	SD	MEAN	SD	MEAN	SD
Cholesterol mmol/L (mg/dl)	5.15 (199.0)	0.71 (33.7)	4.98 (192.5)	0.95 (36.9)	4.85* (187.7)	0.96 (37.1)
LDL mmol/L (mg/dl)	3.27 (126.6)	0.75 (28.9)	3.15 (121.9)	0.88 (34.0)	3.04* (117.6)	0.85 (33.0)
Triglyceride mmol/L (mg/dl)	1.89 (73.0)	0.75 (29.1)	2.00 (77.5)	0.71 (27.6)	1.83 (70.8)	0.62 (23.9)
HDL mmol/L (mg/dl)	1.49 (57.8)	0.13 (4.9)	1.42* (55.0)	0.12 (4.8)	1.45 (56.0)	0.16 (6.0)

*p<0.05; **p<0.01; ***p<0.001 (Compared to high fat diet)¹⁹

Table 5. Mean serum levels of various lipoproteins, LDL particle size, as well as fasting glucose, insulin and c-peptide levels at the end of each dietary period for all subjects combined and by gender.

	Typical American	AHA	High Monounsaturated
Total			
Apo A-I	136.5(15.07)	129.2(14.73)†	132.4(15.99)†‡
Apo A-II	31.1(4.60)	30.3(4.29)†	30.4(4.21)†
Lp(a) 9.2(9.62)	10.1(9.49)†	10.1(9.91)†	
Glucose	81.6(8.89)	80.3(10.99)	81.6(8.43)‡
Insulin	9.9(2.79)	9.6(2.71)	10.2(2.77)‡
C-peptide	1.4(0.41)	1.4(0.40)	1.4(0.42)
LDL-PPD	275.2(4.56)	272.9(6.05)†	273.5(4.85)†
Males			
Apo A-I	131.2(14.70)	122.9(14.94)†	125.5(14.11)†‡
Apo A-II	31.8(4.85)	30.5(4.26)†	30.5(4.25)†
Lp(a) 10.2(10.34)	11.1(10.47)†	11.2(10.88)†	
Glucose	84.0(9.98)	83.8(13.28)	83.4(10.59)
Insulin	9.0(2.72)	9.3(2.80)	9.5(3.14)
C-peptide	1.3(0.34)	1.4(0.35)	1.4(0.39)
LDL-PPD	273.9(4.33)	271.8(5.25)†	272.8(3.69)†‡
Females			
Apo A-I	141.8(13.65)	135.6(11.56)†	139.3(14.86)†‡
Apo A-II	30.4(4.28)	30.2(4.37)	30.3(4.21)
Lp(a) 8.3(8.87)	9.0(8.38)†	9.0(8.83)†	
Glucose	79.1(6.91)	76.8(6.53)	79.7(4.96)‡
Insulin	10.7(2.62)	9.9(2.60)	11.0(2.11)‡
C-peptide	1.5(0.47)	1.5(0.45)	1.5(0.45)
LDL-PPD	276.5(4.48)	273.9(6.64)†	274.1(5.75)†

† Tukey test significantly different from 37%

‡ Tukey test significantly different from AHA

Table 6. Mean and Standard Deviation of the Three Baseline Fasting Serum Glucose, Cholesterol, and triglyceride (mg/dL) for Each Participant in the Absorption Study.

	GLUCOSE		CHOLESTEROL		TRIGLYCERIDE	
	Mean	SD	Mean	SD	Mean	SD
ID=101	87.3	(4.5)	176.3	(9.2)	65.3	(3.2)
ID=102	92.0	(6.9)	211.3	(6.7)	125.7	(18.2)
ID=103	75.7	(1.2)	189.7	(6.7)	54.7	(4.2)
ID=104	99.0	(11.5)	250.7	(16.9)	54.7	(8.1)
ID=105	99.3	(1.2)	220.7	(15.5)	108.0	(16.1)
²¹ ID=106	94.3	(3.1)	272.3	(39.3)	165.0	(45.7)

²¹unpublished data

Table 7. Mean and Standard Deviation of Serum Glucose (mg/dL) by Diet Type and Time since ingestion of the test meal.

	GROUND	WHOLE	OIL
Baseline	90.00 (9.55)	91.33 (9.58)	92.50 (11.61)
1 hour	95.00 (26.12)	90.67 (31.61)	89.00 (16.77)
2 hour	75.67 (14.17)	73.33 (16.5)	76.00 (12.84)
3 hour	76.17 (13.32)	76.83 (12.32)	80.50 (9.87)
4 hour	83.83 (7.25)	80.83 (7.96)	83.33 (7.63)
5 hour	84.17 (6.62)	83.00 (7.56)	83.67 (7.53)
6 hour	84.83 (7.44)	81.33 (5.85)	80.00 (8.22)
7 hour	83.00 (6.66)	86.50 (9.03)	84.00 (8.07)
8 hour	83.83 (8.35)	84.00 (5.93)	82.50 (8.62)

2-way ANOVA p=0.0018 ²²

²²unpublished data

Table 8. Mean and Standard Deviation of Serum Cholesterol (mg/dL) by Diet Type and Time Since Test Diet

	GROUND	WHOLE	OIL
Baseline	216.17 (32.80)	226.33 (51.91)	218.00 (32.02)
1 hour	212.67 (36.33)	223.50 (51.66)	206.83 (31.96)
2 hour	214.67 (36.05)	228.67 (49.85)	202.67 (33.09)
3 hour	211.00 (34.19)	224.33 (42.67)	209.50 (30.74)
4 hour	215.00 (30.48)	222.67 (46.75)	204.00 (30.70)
5 hour	215.83 (29.98)	230.00 (45.88)	211.17 (29.58)
6 hour	213.83 (30.72)	226.67 (48.66)	210.50 (31.35)
7 hour	217.50 (33.92)	227.33 (57.44)	210.50 (34.28)
8 hour	220.00 (32.72)	231.67 (53.87)	211.83 (32.64)

2-way ANOVA p=0.736 ²³

²³unpublished data

Table 9. Mean and Standard Deviation of Serum Triglyceride (mg/dL) by Diet Type and Time Since Test Diet

	GROUND	WHOLE	OIL
Baseline	91.17 (43.74)	91.50 (39.70)	104.00 (59.88)
1 hour	107.83 (66.57)	104.50 (48.90)	132.67 (115.01)
2 hour	141.00 (101.12)	119.50 (54.08)	142.50 (102.47)
3 hour	156.17 (109.70)	127.33 (63.85)	170.83 (96.52)
4 hour	152.33 (104.18)	139.50 (66.61)	181.33 (102.75)
5 hour	142.00 (105.90)	140.33 (52.76)	175.83 (95.86)
6 hour	129.33 (88.64)	126.17 (41.46)	171.83 (103.12)
7 hour	111.00 (77.14)	90.67 (26.43)	147.00 (97.97)
8 hour	103.00 (63.85)	81.17 (19.78)	112.83 (69.21)

2-way ANOVA p=0.035 ²⁴

²⁴unpublished data

Table 10. Mean and Standard Deviation of Serum Glucose (mg/dL) by Diet Type and Participant

	GROUND	WHOLE	OIL
ID=101	77.89 (8.80)	73.11 (16.00)	81.67 (5.48)
ID=102	88.11 (15.32)	91.44 (14.06)	83.11 (8.48)
ID=103	71.11 (5.37)	70.33 (3.16)	67.22 (6.85)
ID=104	94.56 (15.17)	81.67 (8.96)	89.67 (9.85)
ID=105	88.44 (8.56)	90.22 (12.12)	90.78 (8.11)
ID=106	84.22 (7.95)	91.78 (10.86)	88.56 (4.80)

2-way ANOVA p=0.0001 ²⁵

²⁵unpublished data

Table 11. Mean and Standard Deviation of Serum Cholesterol (mg/dL) by Diet Type and Participant

	GROUND	WHOLE	OIL
ID=101	176.56 (7.40)	168.67 (4.74)	183.22 (5.56)
ID=102	205.33 (3.71)	221.78 (8.24)	193.67 (8.80)
ID=103	190.44 (4.75)	198.33 (8.89)	188.00 (5.57)
ID=104	240.33 (9.45)	236.89 (5.99)	247.00 (10.55)
ID=105	214.33 (8.89)	219.67 (7.16)	192.78 (6.78)
ID=106	264.11 (7.87)	315.44 (11.13)	252.00 (5.05)

2-way ANOVA p=0.0001 ²⁶

²⁶unpublished data

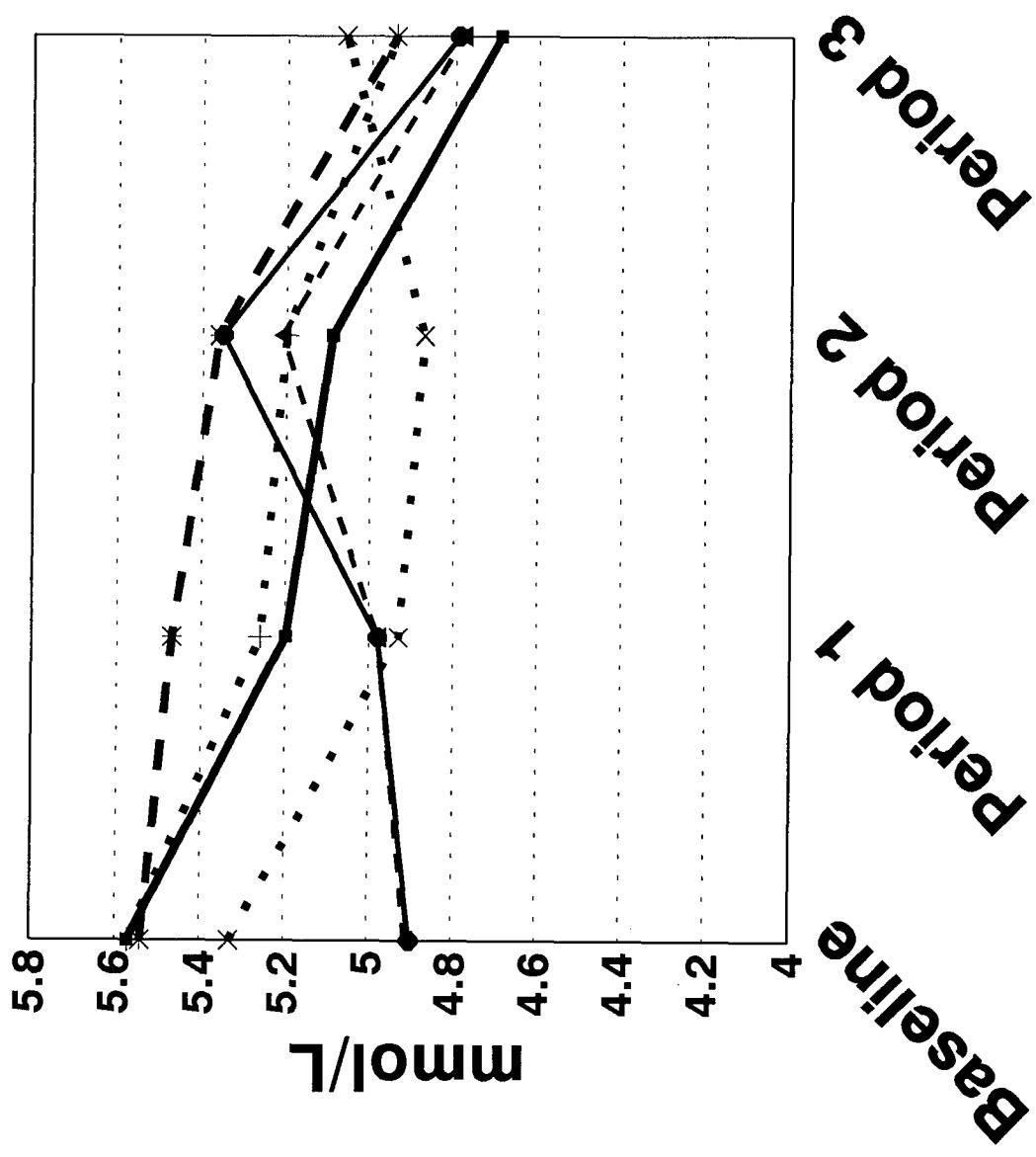
Table 12. Mean and Std of Serum Triglyceride (mg/dL) by Diet Type and Participant

	GROUND	WHOLE	OIL
ID=101	76.00 (11.31)	90.11 (25.94)	124.00 (50.98)
ID=102	150.33 (29.32)	154.33 (29.86)	223.00 (67.59)
ID=103	61.78 (10.20)	67.33 (14.77)	69.44 (20.45)
ID=104	80.89 (12.31)	71.22 (10.87)	59.33 (10.57)
ID=105	102.44 (33.84)	152.56 (61.78)	137.56 (56.74)
ID=106	284.44 (66.93)	144.89 (24.86)	279.22 (50.09)

2-way ANOVA p=0.0001 ²⁷

²⁷unpublished data

Mean Cholesterol for Each of the Six Randomization Groups After Each Dietary Period



BIBLIOGRAPHY OF PUBLICATIONS AND MEETING ABSTRACTS

1. Curb JD, Wergowske G, Dobbs JC, Abbott RD, Huang B. Effects of a Macadamia Nut Based High Monounsaturated Fat Diet on Serum Lipid Levels in Normal Men and Women. Submitted to JAMA.
2. Curb JD, Wergowske G, Dobbs JC, Abbott RD, Huang B. Comparison of Lipid Levels in Humans on a Macadamia Nut Based High Monounsaturated Fat Diet to their Levels on a Moderate Fat Diet and a High Fat "Typical American" Diet. Abstract presented at the American Heart Association Scientific Conference on the Efficacy of Hypocholesterolemic Dietary Interventions, May 3-5, 1995.
3. Curb JD, Wergowske G, Dobbs JC, Abbott RD, Huang B. Comparison of Lipid Levels in Humans on a Macadamia Nut Based high Monounsaturated Fat Diet to their Levels on a Moderate Fat Diet and a High Fat "Typical American" Diet. Presented at the USDA-ARS Western Human Nutrition Center's Nuts and Nutrition Conference at the Presidio of San Francisco on September 28, 1995.

LIST OF ALL PERSONNEL WHO RECEIVED PAY FROM THE CONTRACT

<u>Name</u>	<u>Job Title</u>
Akin, Michael A.	Cook Helper/Food Weigher
Bower, Tony	Student Assistant/Dishwasher
Brown, Patrick	Cook Helper/Food Weigher
Cabiles, Eduardo	Cook Helper/Food Weigher
Chinna, Kyle T.	Cook
Contreras, Tim	Cook Helper
Diaz-Webb, Rita	Clinic Nurse
Garrido, Paul J.	Cook
Hanohano, Gabriel	Clinic Assistant
Handog, Danilo	Student Assistant/Dishwasher
Hein, Evelyn A.	Project Coordinator
Hsia, Liang Ho	Cook Helper/Food Weigher
Huang, Boji	Data Base Systems Designer
Kane, Rod	Cook
McMurtrey, Nancy	Clinic Nurse
Mita, Tom	Cook
Nakamura, Troy	Cook
Nakata, James	Cook
Rabaino, Doreen	Medical Technician
Rabanal, Ofelia	Medical Technician
Remigio, Doris	Medical Technician
Rider, Justin	Telephone Screening Trainer
Rivera, Benedicto	Cook
Sagon, Dionicio	Cook
Shidaki, Ronald	Cook Helper/Food Weigher
Tachino, Nicholas	Nutrition Assistant/Research Assistant
Takamoto, Derek M.	Head Cook
Uyeno, Joyce K.	Study Technician
Velete, Bernard	Cook
Watanabe, Jason	Cook
Williams, Jimmy	Cook Helper/Food Weigher
Williams, Neressa	Cook Helper/Food Weigher
Yeban, Maria	Medical Technician



DEPARTMENT OF THE ARMY

US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y)

21 Apr 97

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCP, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for Grant DAMD17-93-J-3071. Request the limited distribution statement for Accession Document Number ADB210264 be changed to "Approved for public release; distribution unlimited." This report should be released to the National Technical Information Service.
2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322.

FOR THE COMMANDER:

Judie Rinehart
for GARY R. GILBERT
Colonel, MS
Deputy Chief of Staff for
Information Management

*Completed
B.B.
19990609*